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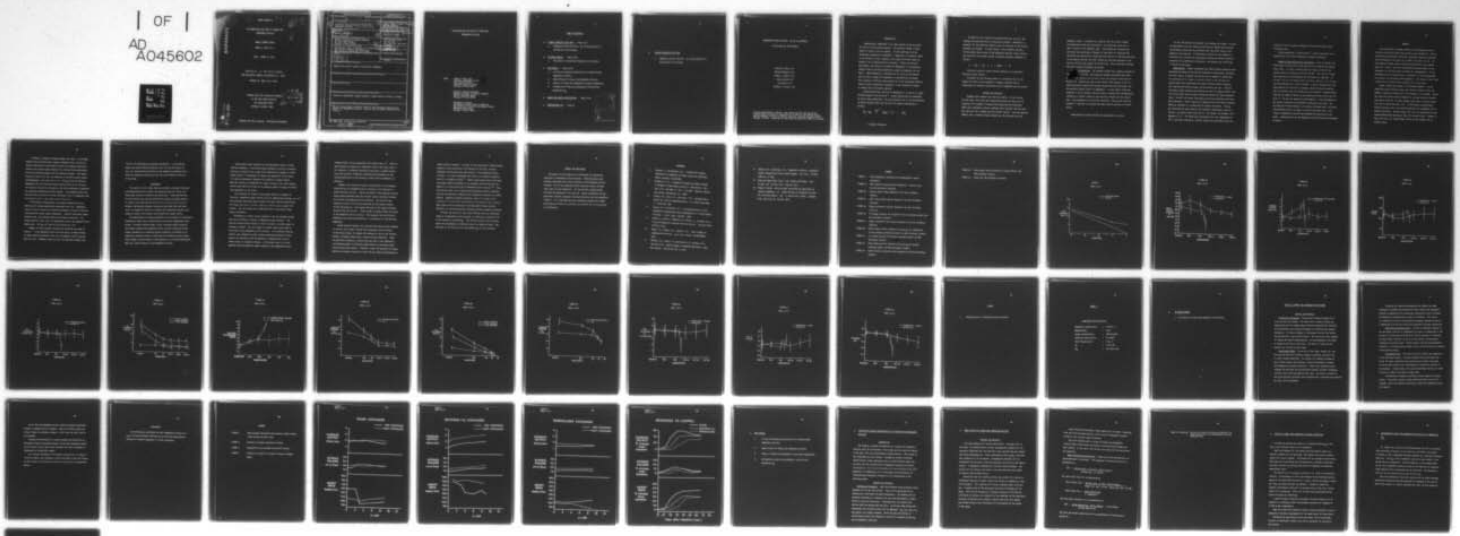

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THE MANUFACTURE AND STUDY OF STROMA FREE HEMOGLOBIN SOLUTION

ANNUAL SUMMARY REPORT

GERALD S. MOSS, M.D.

DATE: AUGUST 15, 1975

SUPPORTED BY: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND, WASHINGTON, D.C. 20314

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Red cell free primate survived on room air when exchange transfused with stroma free hemoglobin solution. Survival time is greater than three (3) hours.

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THE MANUFACTURE AND STUDY OF STROMA FREE HEMOGLOBIN SOLUTION

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A. STUDIES COMPLETED THIS YEAR

1. Hemogolbin-Saline Solution: O_2 and CO_2 Dynamics in The Red Cell Free Primate.

HEMOGLOBIN-SALINE SOLUTION: O_2 and CO_2 DYNAMICS IN THE RED CELL FREE PRIMATE

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INTRODUCTION

Theoretically, hemoglobin is an ideal protein to add to saline for use as a blood volume expander. The molecular weight of hemoglobin is close to that of albumin. Of more importance are the respiratory properties of hemoglobin. Hemoglobin is virtually unique in its ability to bind, transport, and unload sufficient oxygen to maintain life at ambient partial pressures. Within the red cell environment, the affinity of oxygen for hemoglobin is influenced by several factors including temperature, organic phosphates, pH, and pCO_2 [1]. When hemoglobin is removed from its red cell environment, the affinity of oxygen for hemoglobin is substantially increased. This increase in affinity raised doubts as to the respiratory effectiveness of extra erythrocytic hemoglobin, since unloading of oxygen to tissues may be seriously impaired.

A second respiratory function of hemoglobin is to assist in carbon dioxide transport and excretion [2]. Carbon dioxide may be transported by one of three mechanisms. The most important one is via the hydration of carbon dioxide within the red cells to produce bicarbonate as follows:

$$CO_2 + H_2O \overset{C.A.^*}{\rightleftharpoons} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

* Carbonic Anhydrase

In order for this reaction to proceed within the red cell, the hydrogen ion generated must be continually buffered. Hemoglobin is essential for the hydration reaction since it functions as the primary hydrogen ion acceptor. A second system is the carbamino reaction. The N-terminal amino groups of the hemoglobin molecule reacts directly and reversibly with carbon dioxide to produce carbamino compounds as follows:

$$R - NH_2 + CO_2 \rightleftarrows R - NHCOO^- + H^+$$

The third system of carbon dioxide transport is as physically dissolved carbon dioxide.

The object of the following report is to describe the in vivo oxygen and carbon dioxide dynamics in baboons rendered free of erythrocytes by exchange transfusions with a hemoglobin-saline solution.

METHODS AND MATERIALS

Eighteen adult baboons were the test animals. On the morning of each study, the animal was tranquilized within his cage by an IM injection of 0.8 mgm/kg of Phencyclidine Hydrochloride Piperazine. Under local anesthesia, plastic catheters were inserted into the aorta and vena cava via a cutdown over the femoral vessels. Thru the opposite femoral vein, a balloon tipped catheter was flow directed into the

pulmonary artery. A catheter was inserted into the urinary bladder and temperature probe was positioned in the peritoneal cavity thru a small incision in the abdominal wall. The trachea was intubated and the animal was paralyzed by frequent IV injections of d-tubocurarine. The animal was mechanically ventilated with room air in the prone position. In the baseline period, the tidal volume and rate were adjusted to produce an arterial pCO_2 between 35 and 45 mm Hg. These ventilator settings were held constant throughout each study.

Exchange Transfusion: The eighteen animals were randomly assigned to two groups of nine each. One group was exchange transfused with hemo- saline solution and the other group was exchange transfused with Dextran 75*. Dextran was used as the control fluid because it has no known respiratory capabilities beyond transporting dissolved gases, and has molecular weight similar to hemoglobin. In each animal, blood was withdrawn in 50 ml aliquots from the arterial catheter and simultaneously replaced by a similar volume of test solution infused into the femoral vein. The exchange was not strictly isovolumic. Excess test fluid was infused, if required, to maintain the mean arterial pressure at control levels.

* Mean molecular weight 75,000, 6% concentration in saline

At each 10% hematocrit decrement, the exchange was halted. A series of measurements were then made and blood and gas samples were obtained. The exchange transfusion was continued until the animal died or zero hematocrit was achieved. In those that survived the zero hematocrit, sufficient test solution was subsequently infused to maintain baseline pressures for an additional three hours. The animals were sacrificed at the end of the study.

Measurements: Oxygen consumption and carbon dioxide production were determined by measuring the partial pressures of these gases, corrected for water vapor, in samples of room air and in samples of expired gas. The concentration of oxygen and carbon dioxide in the inspired and expired gases were calculated from these corrected partial pressures, the inspired and expired volumes, and the perfect gas laws. Arterial and venous oxygen contents were measured by means of oxygen specific fuel cell (LEX-O_2-CON) which had been calibrated against Van Slyke determinations. Arterial and venous blood gases and pH were measured using standard electrodes. Aortic pressure was measured from the arterial catheter which was attached to a Statham P23 Db pressure transducer. The P_{50} was calculated by least squares, from multiple experimental values on the Po_2 - O_2 content plane using the I.L. 217 system. We assumed a Hill exponent of 2.7. The curves were corrected to pH 7.40, temperature at 37° C, and Pco_2 of 40 mm Hg. Cardiac output was calculated by the Fick

principle from the oxygen consumption and arterial-venous oxygen difference data.

Plasma hemoglobin [3], Methemoglobin [4], sodium, potassium, and phospholipid levels were estimated by standard techniques.

Method of Hemoglobin-Saline Preparation: Units of washed, outdated human red blood cells were lysed by slow freezing and thawing three times. In order to precipitate the stroma, the pH of the pooled lysate was reduced to 5.8 with .2N HCL. The red cell stroma were separated by centrifugation for 20 minutes at 3,000 rpm. To minimize acid induced methemoglobin formation, the pH of the solution was then elevated to 7.0 with .2N NaOH. A cation exchange resin was then added to the solution to exchange sodium for excess potassium. The ion exchange resin was removed by centrifugation. Aluminum hydroxide gel was added to absorb an anticoagulant evolved during red cell lysis [5]. The gel was removed by centrifugation. Final adjustment of pH, sodium, potassium and hemoglobin concentration was made. The solution was filtered through a 0.22 micron millipore and a .01 micron Sietz-Berkfeld filter to render it sterile and stroma free. Five liters of hemoglobin solution were produced the day prior to each study. Characteristics of the hemoglobin-saline solution are presented in Table 1.

RESULTS

The nine animals exchange transfused with hemoglobin-saline solution survived the three hour period at zero hematocrit. During the exchange transfusion, the total circulating hemoglobin declined from an average of 13.8 gms % (± .8 SEM) to 6.3 gms % (± .4). All nine Dextran treated animals died when the hematocrit was reduced below 6%. Figure 1 shows the relationship between hematocrit and hemoglobin for the two groups corrected for methemoglobin. In the hemoglobin-saline solution group, a hematocrit of zero corresponded to 5.8 gms % of circulating free hemoglobin. The volume of test fluid infused was not significantly different in the two groups.

Figure 2 shows the changes in circulatory dynamics. In the hemoglobin-saline solution group, mean arterial pressure (Fig. 2a) showed no important changes until zero hematocrit was reached. During the three hour period at zero hematocrit, the mean arterial pressure declined by 25 mm Hg. No major changes were noted in cardiac output (Fig. 2b) or heart rate (Fig. 2c). In the Dextran treated group, arterial pressure (Fig. 2a) declined progressively throughout the exchange transfusion. Cardiac output (Fig. 2b) rose substantially until extreme hemodilution developed, then fell precipitiously. Changes in heart rate (Fig. 2c) showed trends similar to the changes seen in cardiac output.

In Figure 3, changes in oxygen dynamics are shown. In the hemoglobin-saline solution group, oxygen consumption (Fig. 3a) did not deviate significantly from baseline during the exchange transfusion. Arterial and venous oxygen contents (Fig. 3b) declined synchronously while the difference between the two remained constant. The oxygen extraction ratio (Fig. 3c), the fraction of available oxygen removed from hemoglobin, rose from .24 to .51. The P_{50} of the circulating hemoglobin (Fig. 3d) declined from a baseline value of 32 mm Hg to 13.5 mm Hg at the end of the study as the intra erythrocytic hemoglobin was replaced by hemoglobin-saline solution. The pulmonary artery Po_2 declined from 51 mm Hg to 17 mm Hg reflecting a fall in hemoglobin mass and leftward shift in the oxygen dissociation curve.

In the Dextran treated group, the oxygen consumption did not decline until extreme hemodilution developed (Fig. 3a). Hemoglobin levels as low as 3.8 gms % or equivalent hematocrit values of 10% were associated with normal oxygen consumption. Arterial and venous oxygen contents (Fig. 3e) declined during the exchange transfusion. The oxygen extration ratio (Fig. 3c) approached unity as the hematocrit fell toward zero. The P_{50} fell from 32 to 26 mm Hg (Fig. 3f).

Changes in carbon dioxide transport and excretion are shown in Figure 4. In the hemoglobin-saline solution group, no major changes in carbon dioxide production (Fig. 4a) or pulmonary artery Pco_2 were seen Fig. 4b). Pulmonary artery pH (Fig. 4c) declined slightly from

7.45 to 7.31 following the exchange transfusions. In the Dextran group, the carbon dioxide production (Fig. 4a) and the venous pH (Fig. 4c) declined precipitiously as the hematocrit approached zero, while the pulmonary artery Pco_2 (Fig. 4b) rose slightly at the end of the study.

DISCUSSION

The results of this study show that primates, exchange transfused with hemoglobin-saline solution and breathing room air, survive for three hours without circulating red blood cells. These observations are not entirely new, but the quantitative aspects of oxygen dynamics in animals given hemoglobin-saline solution have not been described. Further, the Dextran exchange transfusion data shows that red blood cells, at hematocrits above 6 %, perform the respiratory function well enough to sustain life without any extracellular oxygen carrier.

The maintainence of oxygen consumption in the presence of progressive normovolemic anemia may be explained by two possible compensatory mechanisms - increased cardiac output, and/or increased oxygen unloading. The animals treated with hemoglobin-saline solution maintained normal oxygen consumption by increasing oxygen unloading, as manifest by increased O_2 extraction ratios, rather than by increasing cardiac output. These changes occurred despite a 60% reduction in circulating hemoglobin mass and a large increase in the hemoglobin affinity.

These results focus attention on the physiologic effects of high affinity hemoglobin. Persistent oxygen unloading in spite of increased affinity, as noted in this study, may be explained by changes in tissue oxygen tension. The movement of oxygen from hemoglobin to the tissue is a passive phenomenon, dependent on gradients in oxygen tension. When the affinity of hemoglobin for oxygen increases, the tissue oxygen tension must decline in order for an adequate supply of oxygen to diffuse from hemoglobin to the tissue.

This principle of reduced tissue oxygen tension in response to increased hemoglobin oxygen affinity was also demonstrated by Riggs, et. al.[6] They exchange transfused anemic monkeys with high affinity banked blood. No changes were seen in cardiac output or arterial venous oxygen differences. The only change was a significant fall in the mixed venous oxygen differences.

Differences in cardiac output response in the two treatment groups may also be related to changes in hemoglobin oxygen affinity. The Dextran treated animals showed an initial increase in cardiac output in response to anemia. No such changes in cardiac output were noted in the animals given hemoglobin-saline solution. It is possible that the animals given hemoglobin-saline solution developed myocardial tissue hypoxia and therefore could not develop an increased heart rate or stroke volume in response to anemia. On the other hand, it is also possible that the degree of anemia produced in the hemoglobin-saline

treated animals did not appreciably fall below 6 gms. % [7]. There is good evidence to suggest that hemoglobin levels lower than 6 gms. % are necessary to stimulate consistant elevations in cardiac output. Thus, more specific information regarding the performance of the stressed myocardium in the presence of high affinity hemoglobin is necessary.

Another issue requiring further clarification is the unloading characteristics of hemoglobin-saline solution in the presence of normal affinity red cells. Kaplan and Murthy [8] have measured arterial venous oxygen gradients when the vascular system contained a mixture of red cells and hemoglobin-saline solution. The red cells and hemoglobin-saline solution were separated and the oxygen contents of each was measured. They showed that most of the oxygen unloading occurred from the red cell. Little change in oxygen content was noted in the hemoglobin-saline solution. This suggests that low affinity hemoglobin may unload preferentially in the presence of high affinity hemoglobin.

Carbon dioxide transport and excretion data have not been reported in red cell free animals treated with hemoglobin-saline solution. In the present study, no changes were observed in any of the relevant indices, including venous Pco_2 or carbon dioxide production. Since no significant elevation in venous Pco_2 was seen in zero hematocrit, an increased amount of dissolved carbon dioxide in the venous blood could not have been present. Therefore, either the hydration of carbon dioxide or carbamino formation or both were the important mechanisms of

carbon dioxide transport. In order for the hydration of carbon dioxide reaction to have persisted in the absence of red cells, carbonic anhydrase activity must have been present in the hemoglobin-saline solution. It is well documented that there is no carbonic anhydrase activity in mammalian plasma [9]. Recent unpublished studies have shown that human carbonic anhydrase is an extremely resilient protein and appears to survive processing in hemoglobin-saline solution [10]. Thus, the solution used in our study may have possessed carbonic anhydrase activity and therefore promoted extra erythrocytic hydration of carbon dioxide. Regarding carbamino formation, there is no data in the literature or in our study concerning the resilience of the N-terminal amino groups during processing of the hemoglobin-saline solution. Therefore, we can only speculate regarding possible carbamino formation.

Finally, the results of this study indicate that the respiratory capacity of hemoglobin-saline solution is sufficient to maintain life in the absence of erythrocytes. The clinical value of this solution awaits the development of additional data in a number of areas. One such area is the definition of the optimum P_{50} for this solution.

SUMMARY AND CONCLUSION

The object of this study was to investigate the respiratory properties of hemoglobin-saline solution. Eighteen baboons were exchange transfused with either 6% Dextran of 6% hemoglobin-saline solution. All of the hemoglobin-saline solution animals survived three hours at zero hematocrit. All the Dextran treated animals died when the hematocrit fell below 6%. Oxygen consumption and CO_2 production remained unchanged from baseline during the zero hematocrit interval. It is concluded that the respiratory capability of hemoglobin-saline solution is sufficient to maintain life in the absence of erythrocytes.

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LEGENDS

FIGURE 1. Total hemoglobin (corrected for methemoglobin) versus hematocrit.

FIGURE 2a. Mean arterial pressure versus hematocrit. Vertical bars are 95% confidence intervals.

FIGURE 2b. Cardiac output versus hematocrit with 95% confidence interval.

FIGURE 2c. Heart rate plotted against hematocrit and 95% confidence intervals.

FIGURE 3a. Oxygen consumption against hematocrit and 95% confidence intervals.

FIGURE 3b. A-V oxygen contents for hemoglobin-saline exchanged animals and 95% confidence intervals.

FIGURE 3c. Oxygen extraction ratio against hematocrit and 95% confidence intervals.

FIGURE 3d. Mixed venous partial pressure of O_2 and P_{50} for hemoglobin-saline exchange transfused animals and 95% confidence interval.

FIGURE 3e. A-V oxygen contents for Dextran exchanged animals and 95% confidence interval.

FIGURE 3f. Mixed venous partial pressure of O_2 and P_{50} for Dextran exchanged animals and 95% confidence interval.

FIGURE 4a. Carbon dioxide production versus hematocrit and 95% confidence interval.

FIGURE 4b. Mixed venous partial pressure of carbon dioxide and 95% confidence interval.

FIGURE 4c. Venous pH, 95% confidence intervals.

FIGURE 1

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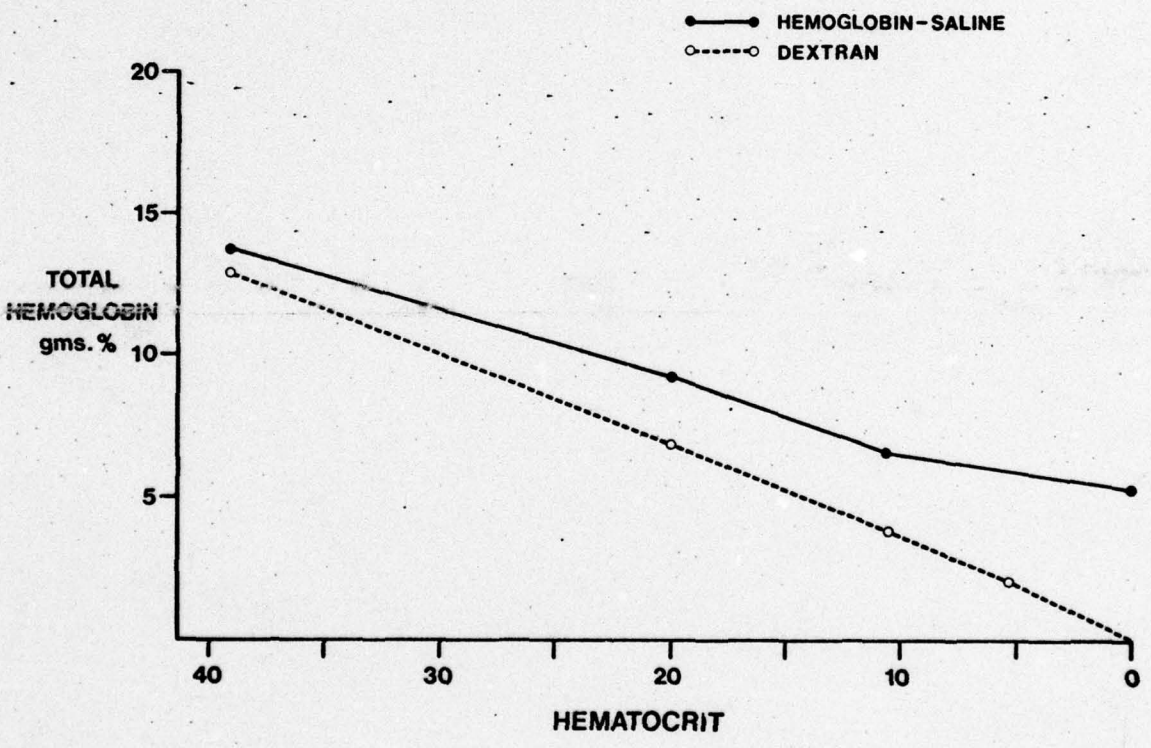

FIGURE 2a

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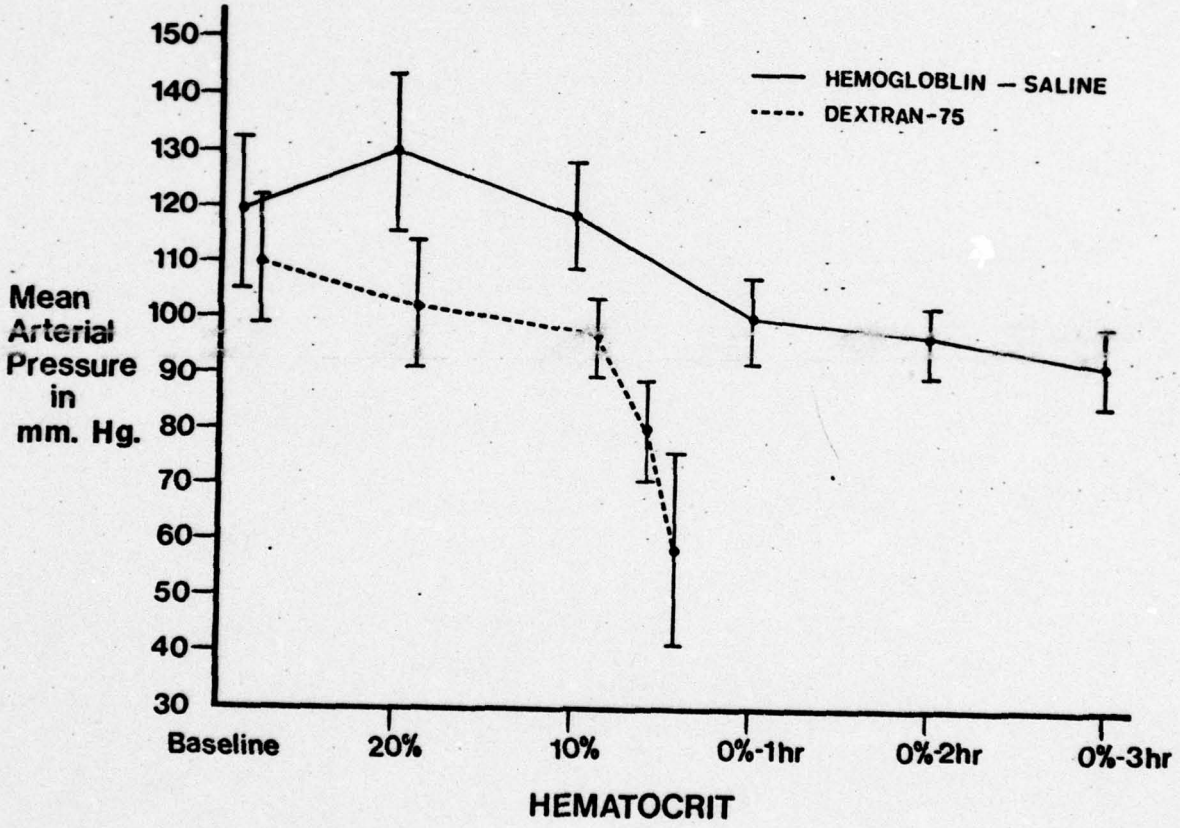

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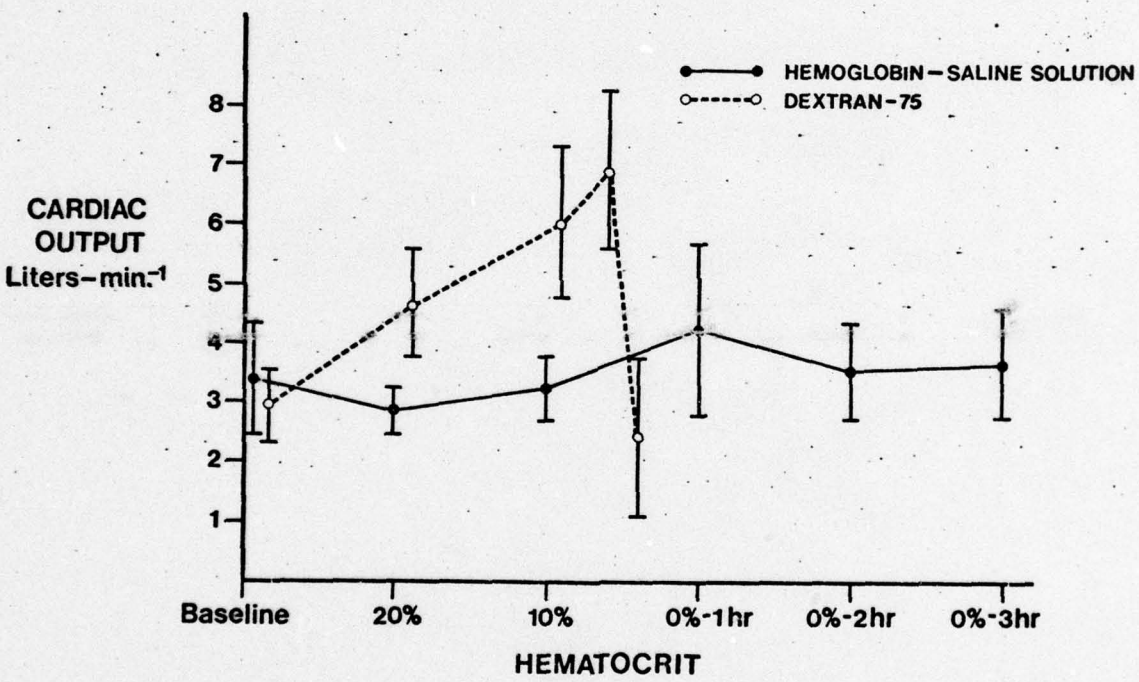

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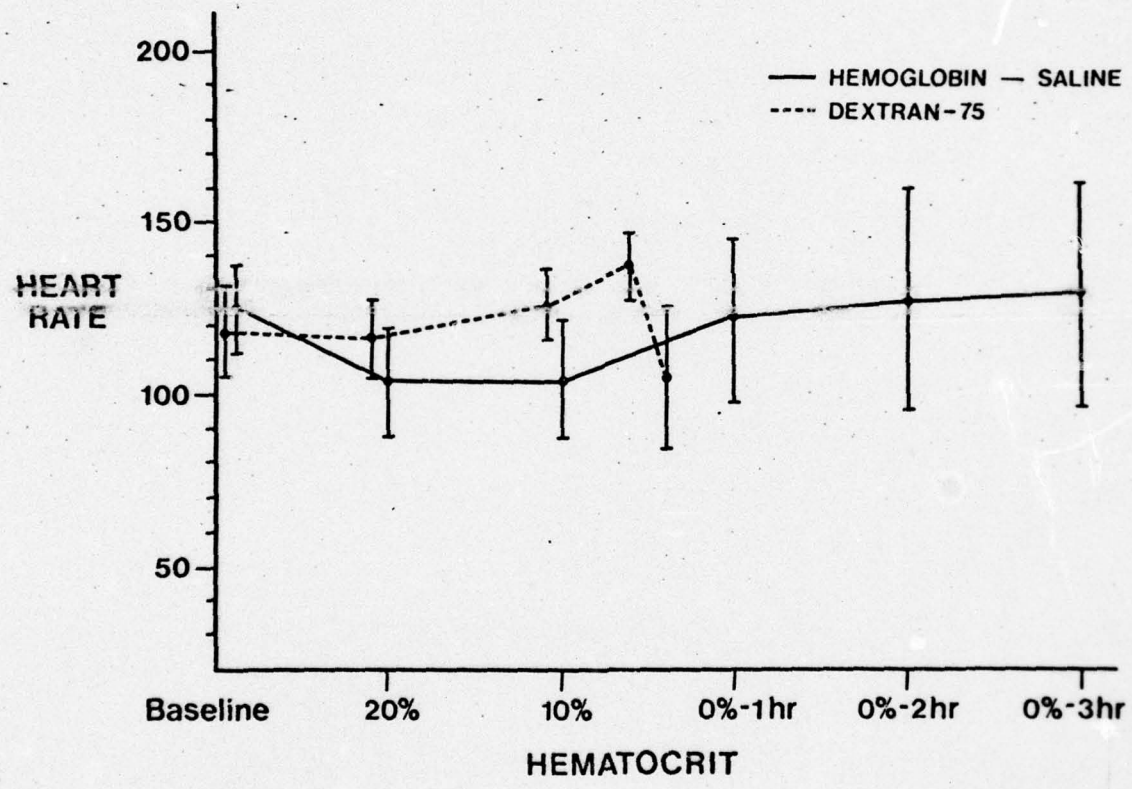

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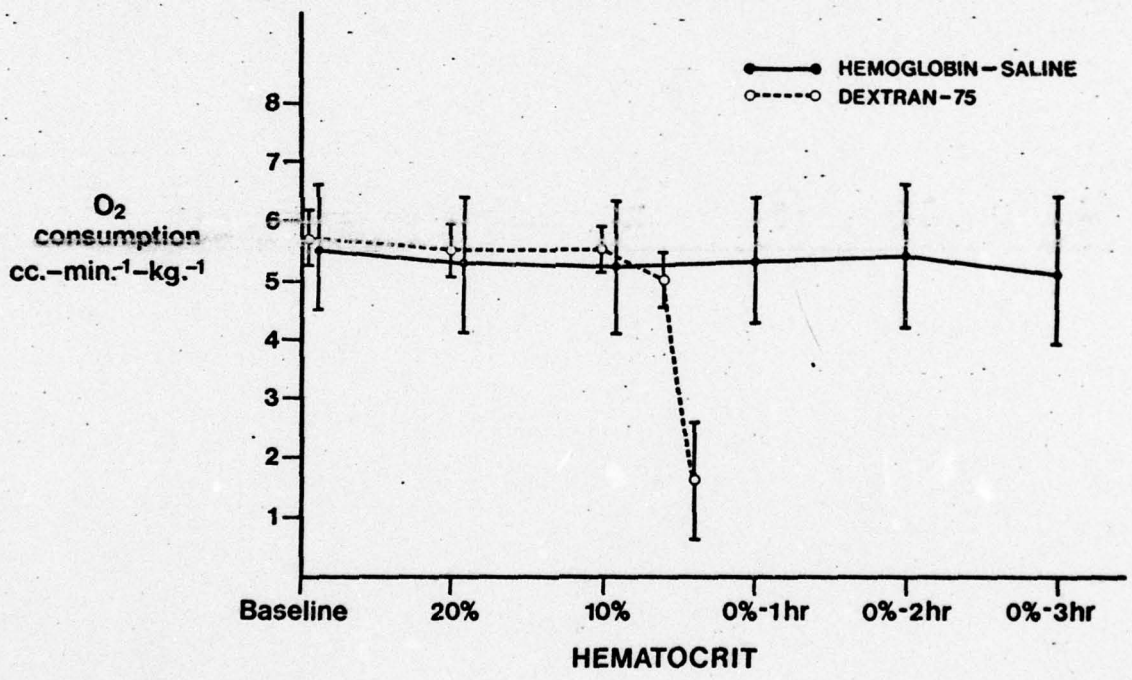

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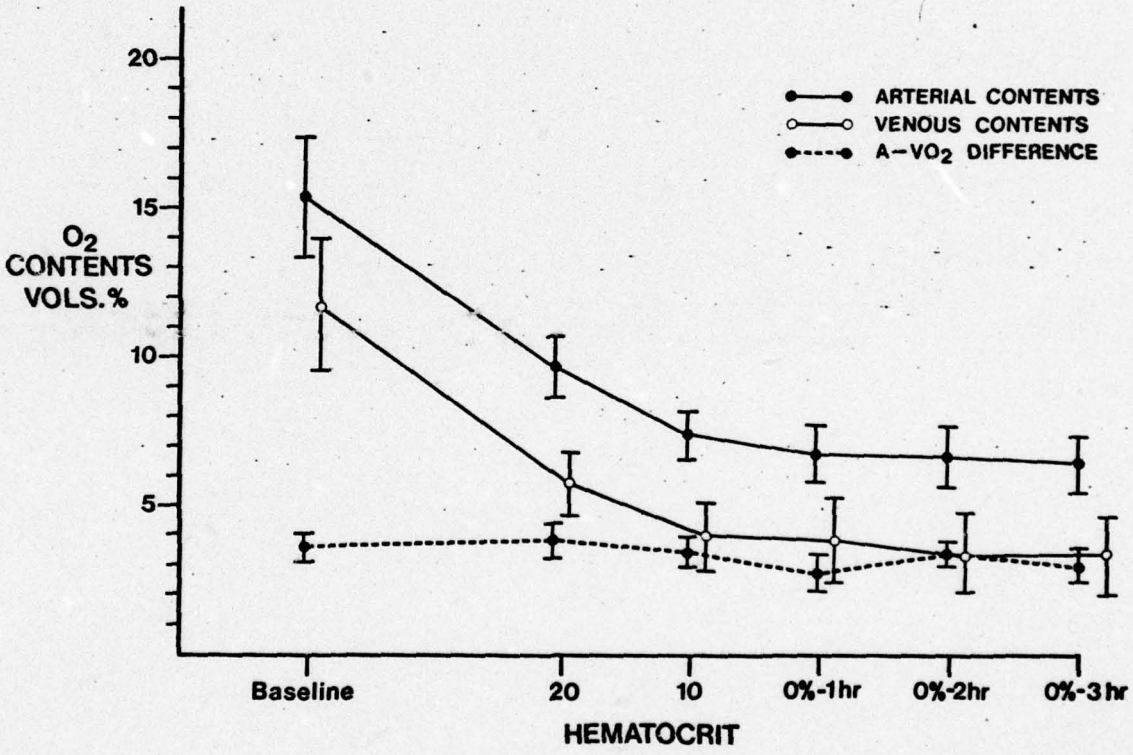

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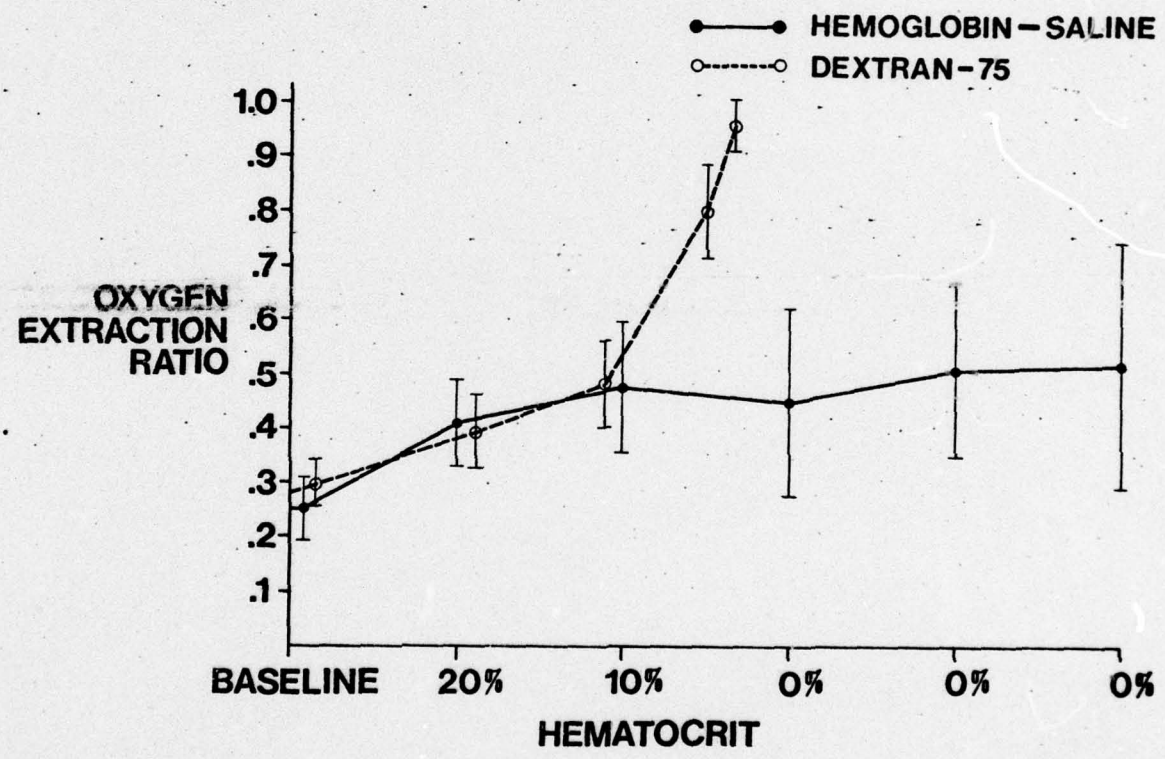

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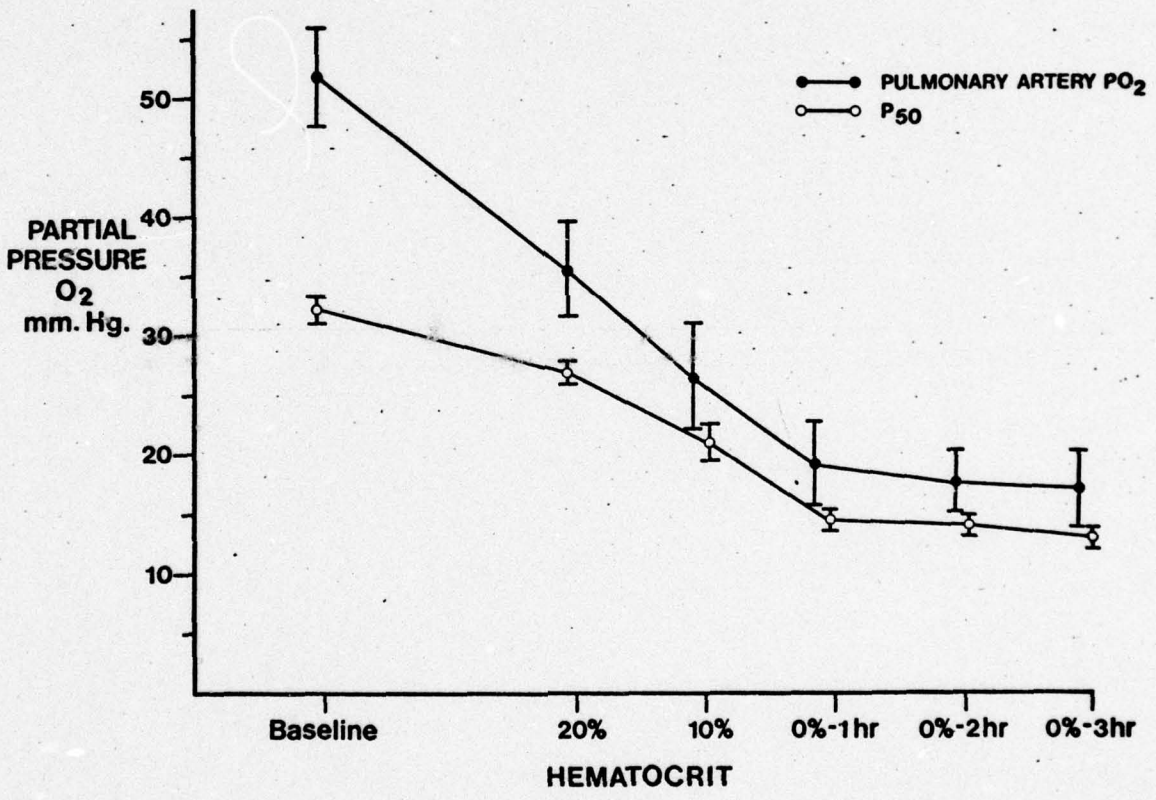

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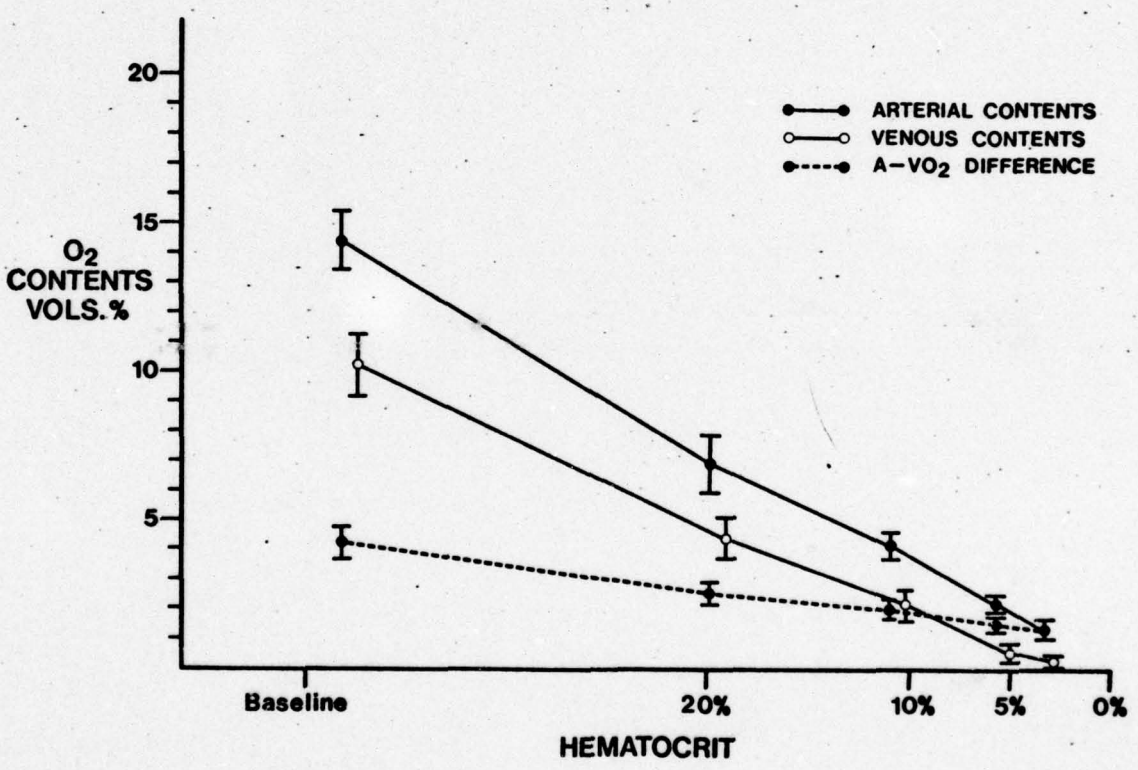

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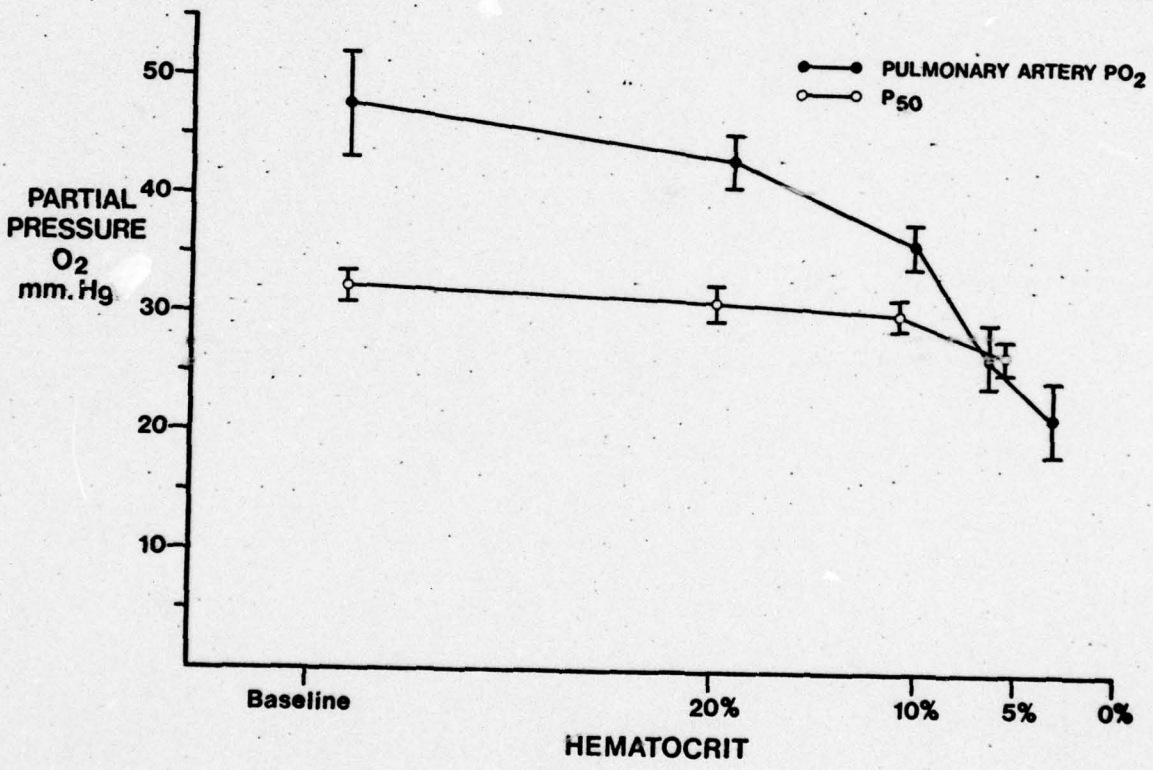

FIGURE 4a

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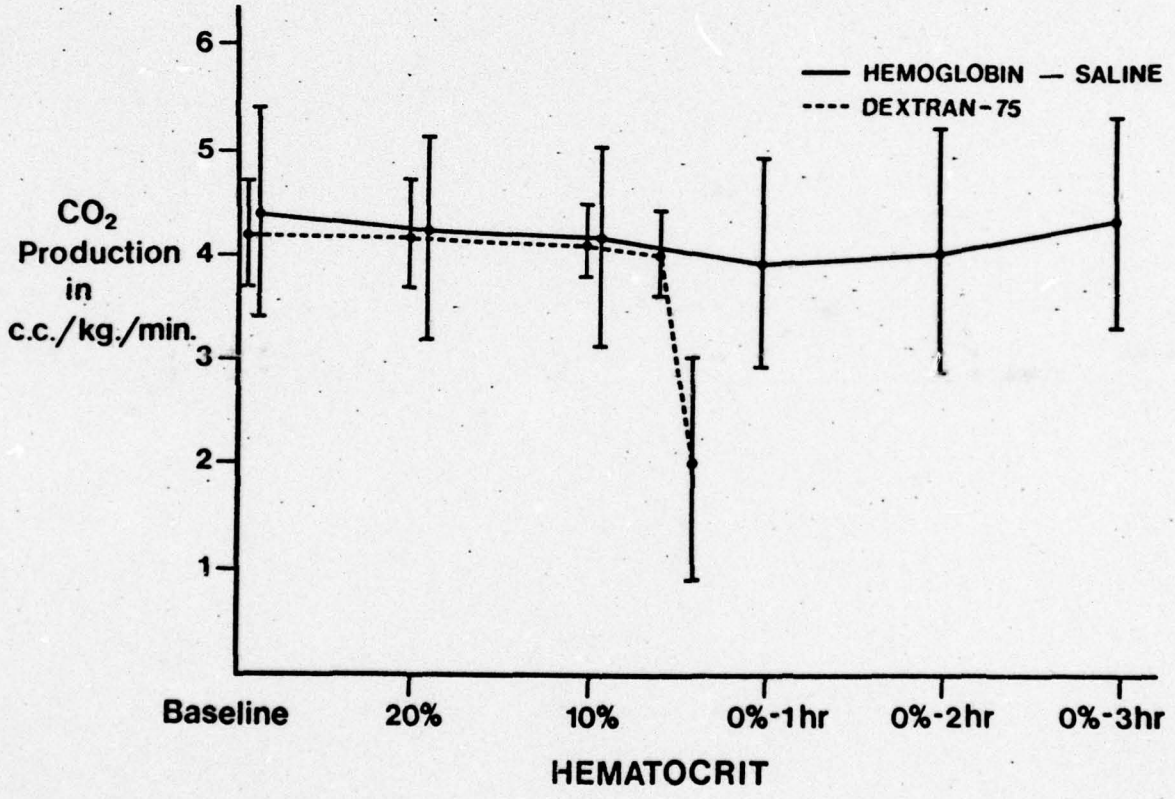

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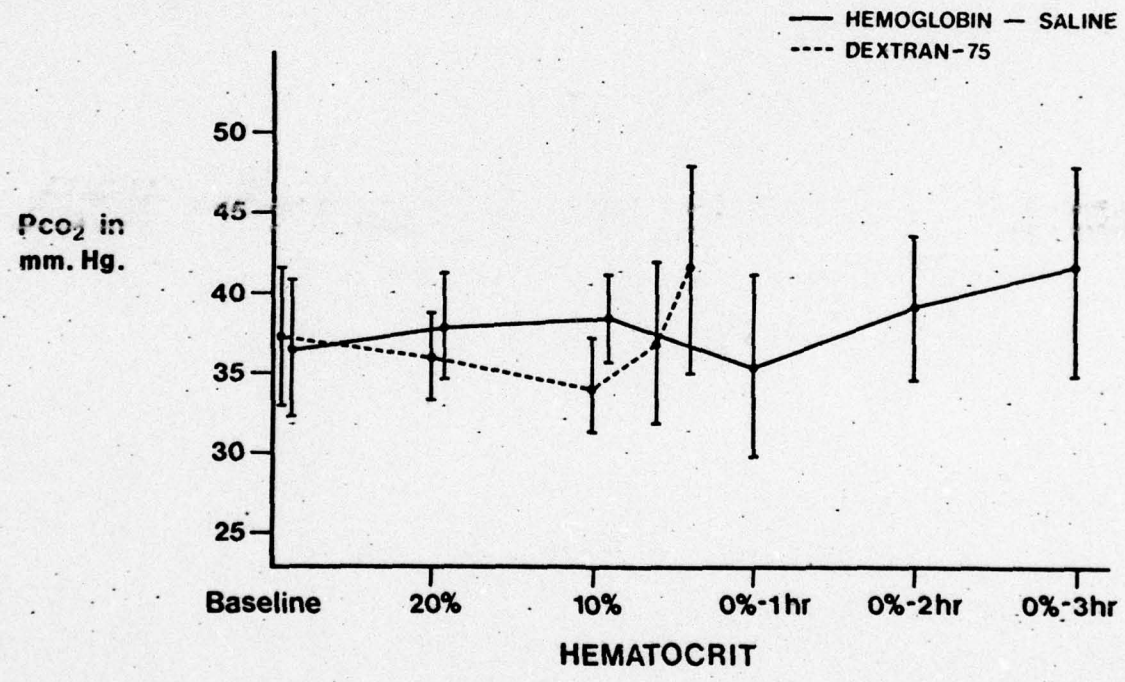

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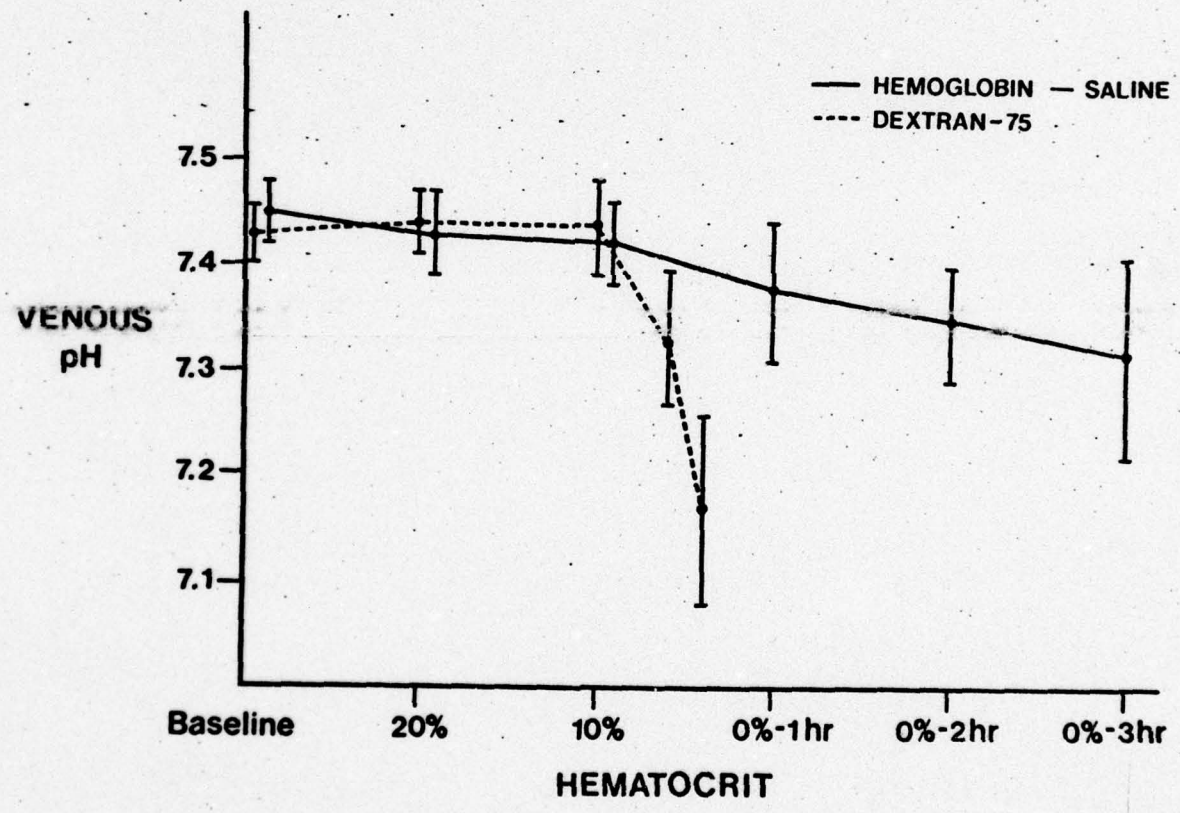


TABLES

1. Characteristics of hemoglobin-saline solution.

TABLE I

HEMOGLOBIN-SALINE SOLUTION

Hemoglobin Concentration	=	6-9 gms. %
Methemoglobin	=	2-4%
Sodium Concentration	=	140-150 mEq/L
Potassium Concentration	=	3-4 mEq/L
Total Phospholipids	<	1 mg. %
pH	=	7.35-7.45
P_{50}	=	14.0 mm of Hg.

B. ON GOING STUDIES

1. The Effect of Stroma Free Hemoglobin on the Heart.

EFFECT OF STROMA FREE HEMOGLOBIN ON THE HEART

METHODS AND MATERIALS

Preliminary Procedures: Fifteen adult baboons weighing 12 to 30 kg are the test animals. One week prior to study, animals are tranquilized with 0.7 mgm/kg phenylcyclidine hydroxhloride (Sernylan, Phillip-Roxanne) and a median sternotomy is performed under general anesthesia. A silastic catheter is introduced into the left atrium and secured with a purse string suture. The free end of this catheter is ligated and buried subcutaneously. An electromagnetic flow probe is placed on the root of the aorta. The wound is closed and the animals are returned to their cages.

Experimental Model: On the day of the study, animals are tranquilized and the atrial catheter exposed, aspirated, and free flow or atrial blood established. The animals are randomly assigned to one of three groups; sham exchange, Dextran-75 exchange or stroma free hemoglobin exchange transfusion. Under local anesthesia groin cutdowns are performed and polyethylene catheters (PE-240) introduced into the aortic arch and superior vena cava. The animal is placed in the prone position, paralyzed with d-tubocurarine, ventilated and arterial pH, Pco_2, and Po_2 adjusted.

Following the baseline measurements the animals are sham exchanged or exchange transfused with either stroma free hemoglobin solution or Dextran-75 on a volume per volume basis until a decrease of hemoglobin concentration to 6 gms % is achieved. This occurs at a hematocrit of 18% in the Dextran-75 exchange transfusion and at a hematocrit of 0% in the stroma free hemoglobin exchange transfusion.

Ventricular Function Curves: In order to determine whether or not the heart's ability to compensate for stress is compromised in the absence of circulating red blood cells, atrial pressure is increased by rapid venous infusions of 200 cc of the animals own previously withdrawn circulating fluid. Stroke volume, from the electromagnetic flowmeter, is plotted against change in left atrial pressure to generate Frank-Starling curves.

Preliminary Data: The results of pilot studies are summarized in the following figures. The sham exchange figure represents the effect of simply withdrawing and reinfusing the animals own blood. As can be seen, there are no cardiologically significant effects of the maneuver. Interestingly, the classic Bainbridge response to rapid infusion is shown in the graph of Heart Rate.

The Dextran-75 exchange transfusion yields somewhat different results. The cardiac output, stroke volume and heart rate are all elevated after the exchange transfusion (circulating hemoglobin level of 6 gms %).

We see that the hemoglobin-saline solution exchange transfusion presents a somewhat similar response. Again the cardiac output and stroke volumes are elevated, however, in this case the heart rate is not elevated.

Although the differences in response between the Dextran-75 or hemoglobin exchange transfused animals and the sham exchanged animals are not large, we feel they fairly represent the heart's attempt to compensate for normovolemic anemia.

As a further challenge all the animals received an I.V. bolus of .02 mg of Isuprel, the responses to which are shown in the last figure. As can be seen, all the animals respond to the dose in an appropriate manner.

CONCLUSION

Our preliminary conclusions are that hemoglobin solution at a P_{50} of 14 mm Hg provides sufficient O_2 to allow the myocardium to continue to function adequately in stress situations.

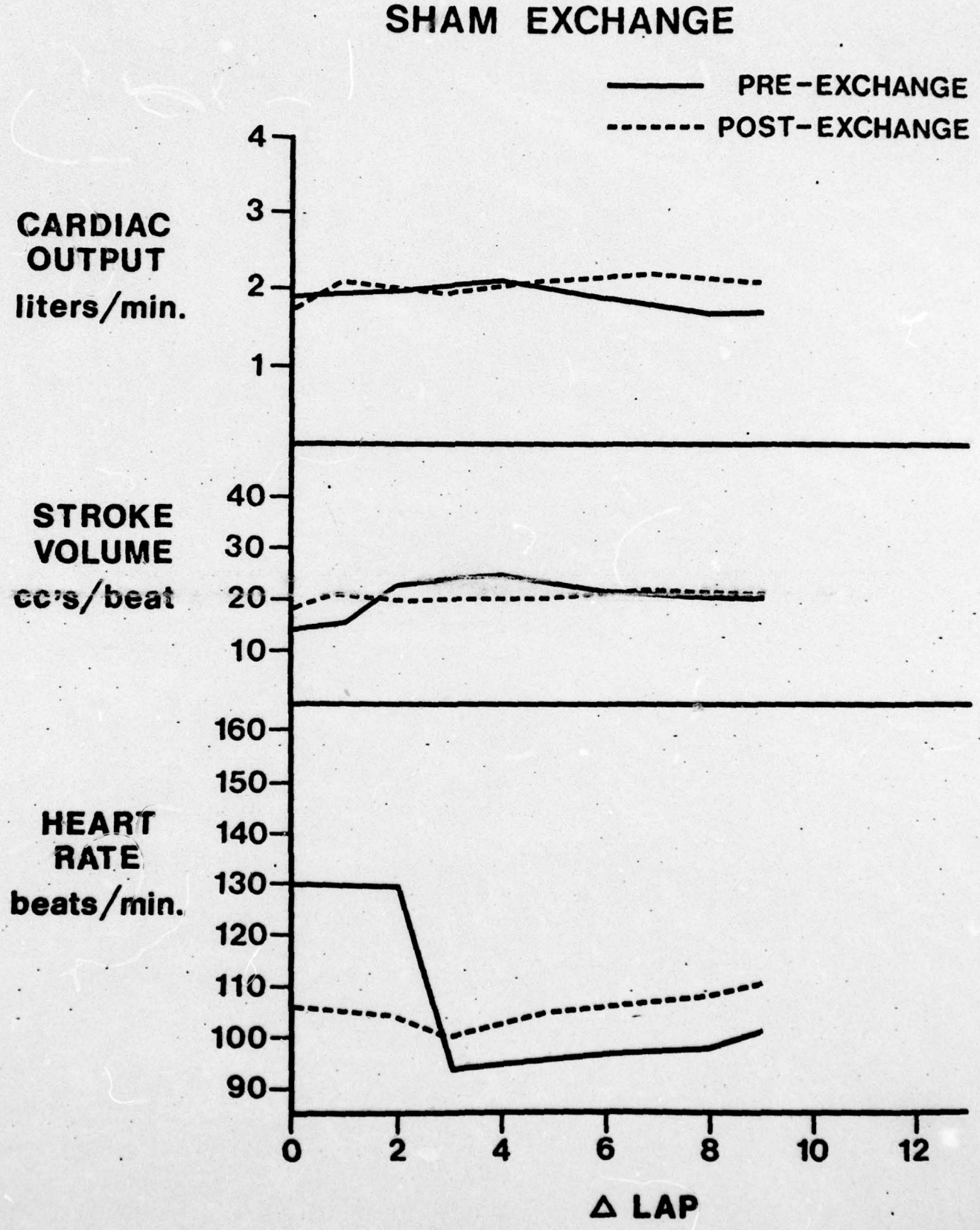
LEGENDS

FIGURE 1. Sham exchange transfusion data showing cardiac output, stroke volume and heart rate.

FIGURE 2. Dextran-75 exchange transfusion indices.

FIGURE 3. Hemoglobin-saline exchange transfusion indices.

FIGURE 4. Response to Isuprel for animals from each exchange group.


SHAM EXCHANGE
PRE-EXCHANGE
POST-EXCHANGE
CARDIAC OUTPUT
liters/min.
4
3
2
1
STROKE VOLUME
cc's/beat
40
30
20
10
HEART RATE
beats/min.
160
150
140
130
120
110
100
90
0
2
4
6
8
10
12
Δ LAP

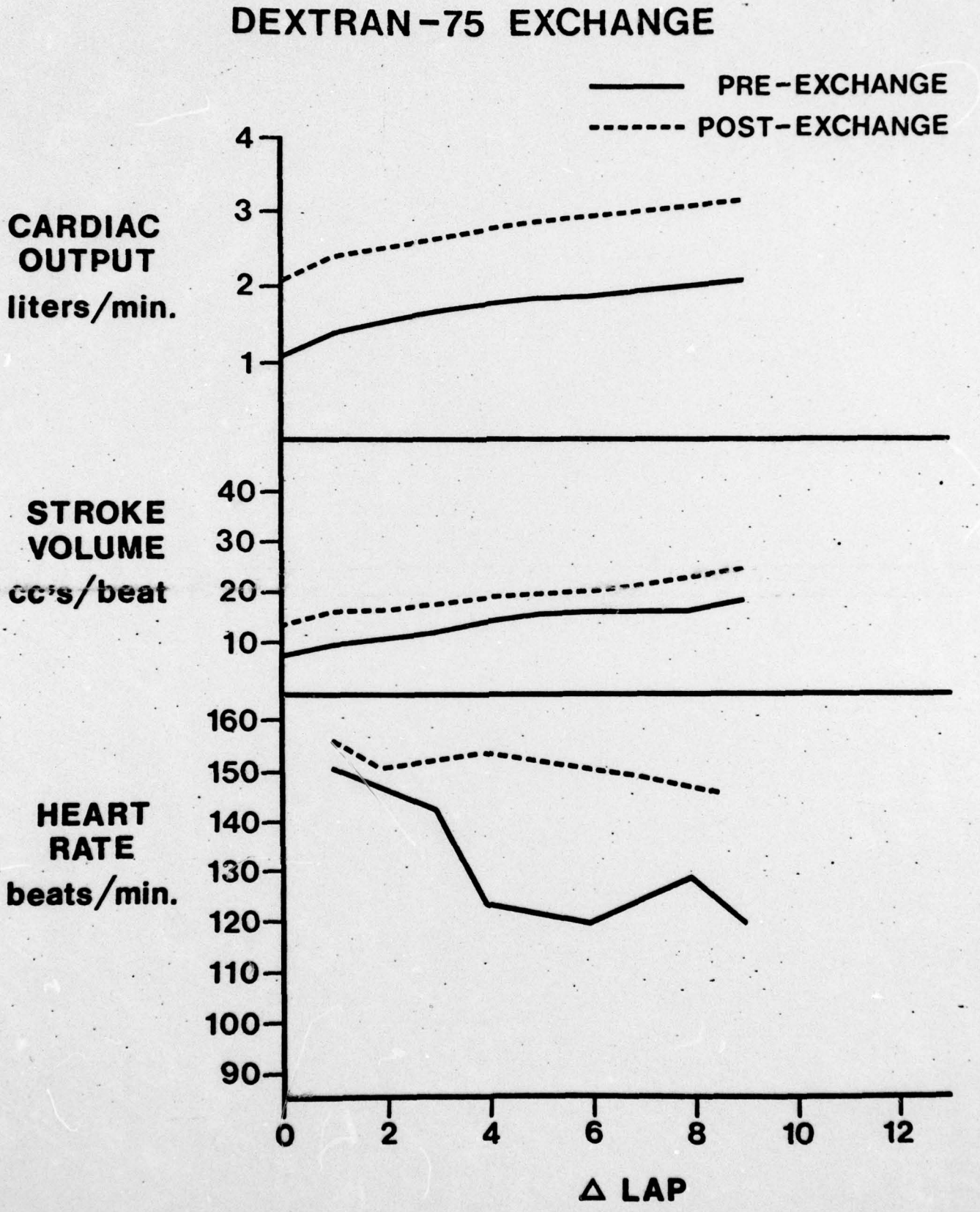

DEXTRAN-75 EXCHANGE
PRE-EXCHANGE
POST-EXCHANGE
CARDIAC OUTPUT
liters/min.
4
3
2
1
STROKE VOLUME
cc's/beat
40
30
20
10
HEART RATE
beats/min.
160
150
140
130
120
110
100
90
0
2
4
6
8
10
12
Δ LAP

FIGURE 3
MOSS, et.al.

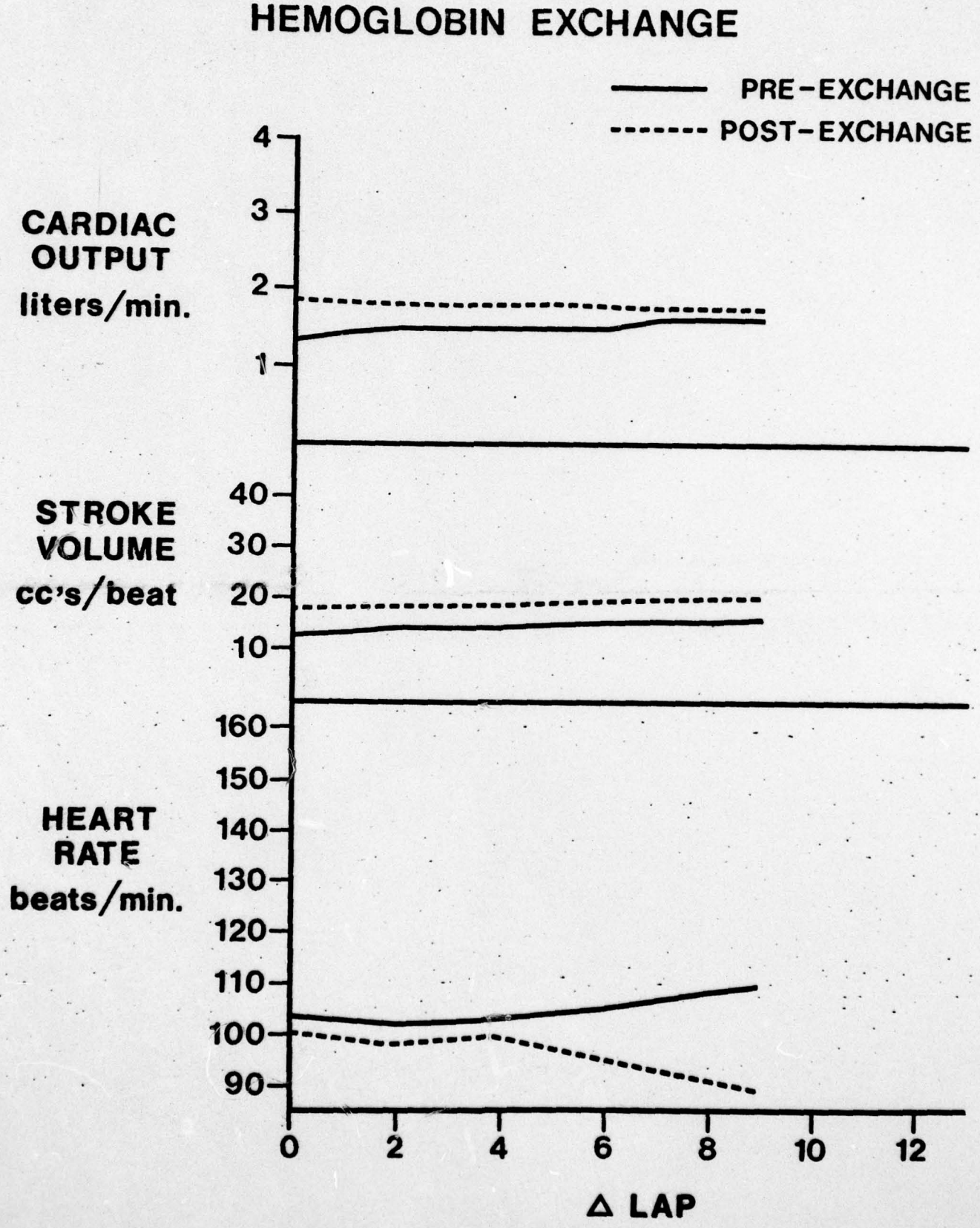
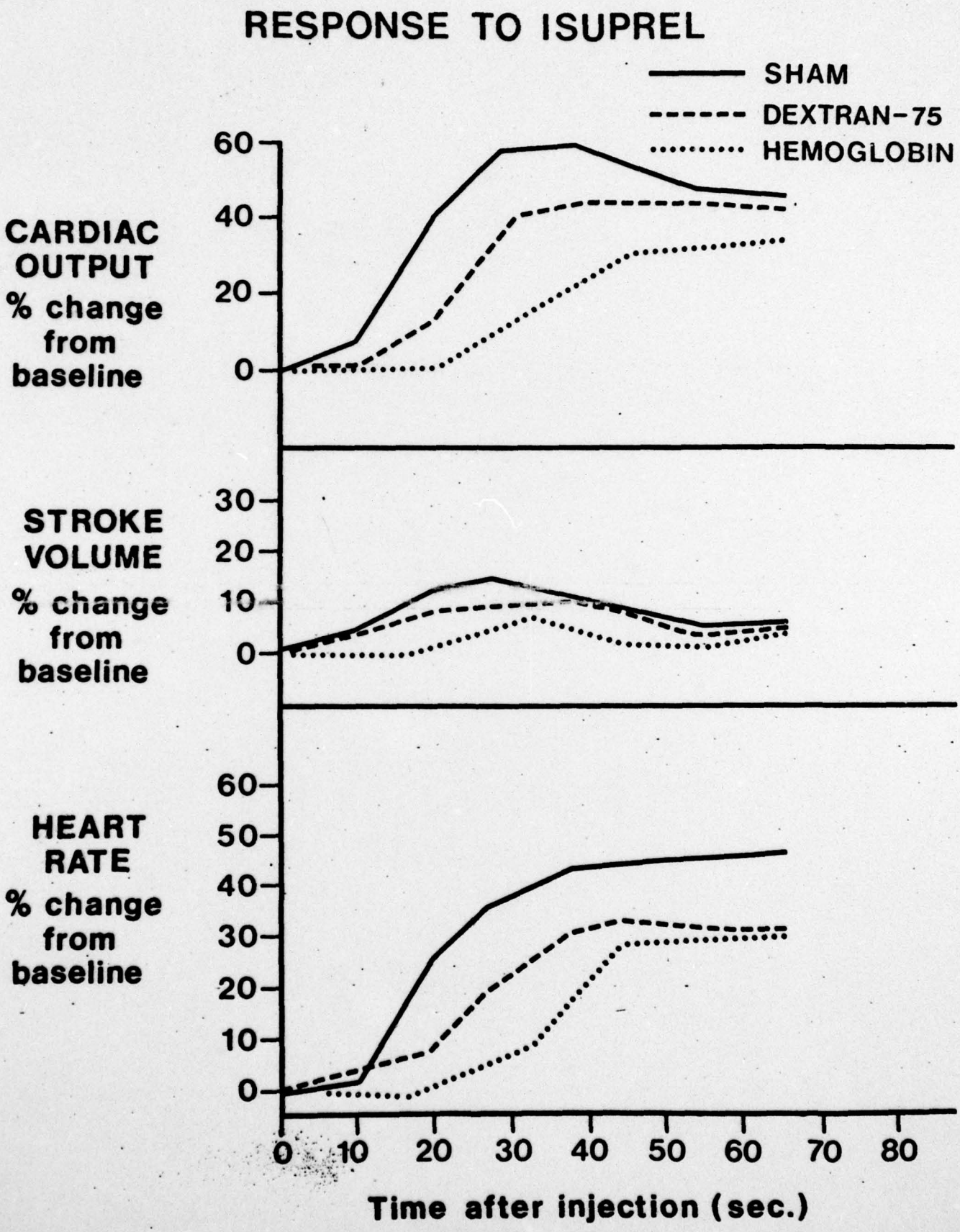


FIGURE 4
MOSS, et.al.



C. NEW STUDIES

1. In Vivo Off-Loading Characteristics of Reconstituted Hemoglobin Solution.

2. Renal Effects of Stroma Free Hemoglobin Solution.

3. Effect of Stroma Free Hemoglobin on the Brain Oxygenation.

4. Collaborative study using hemoglobin solution with normalized P_{50}.

1. IN VIVO OFF-LOADING CHARACTERISTICS OF RECONSTITUTED HEMOGLOBIN SOLUTION

INTRODUCTION

The shipment, storage and handling of a stroma free hemoglobin solution would be facilitated if its weight and bulk could be reduced. In the past, this was accomplished by lyophilization. The success of this technique has been limited. Although we intend to evaluate lyophilization, other techniques such as ultrafiltration might also be useful for the concentration of hemoglobin solution and require investigation. Once the best technique for concentrating stroma free hemoglobin is determined, the in vivo oxygen carrying capacity of "reconstituted" hemoglobin solution will be investigated in the following manner.

METHODS AND MATERIALS

Preliminary Procedures: Adult male baboons having bilateral groin cutdowns will be the test animals. They will be paralyzed with d-tubocurarine, ventilated and kept normothermic. The animals will be exchange transfused to a hematocrit of zero and maintained at a hematocrit of zero for three hours. Following this, the animals will receive their own washed shed red cells. At various times during the experiments the following values will be obtained: P_{50}, Po_2, Pco_2, pH, and jugular vein oxygen contents. From this data the ability of reconstituted stroma free hemoglobin solution to transport O_2 and CO_2 can be adequately evaluated.

2. RENAL EFFECTS OF STROMA FREE HEMOGLOBIN SOLUTION

METHODS AND MATERIALS

Five adult baboons will be the test animals. One week prior to the study, siliconized heparin-filled, polyethylene catheters will be surgically implanted into the left renal vein, and the free end ligated and buried subcutaneously. After convalescence from surgery, the renal vein catheter will be re-exposed. Polyethylene catheters will be introduced into the aortic arch and suprarenal vena cava via the femoral vessels. A suprapubic cystotomy will provide urinary drainage. The animals will be loosely restrained in the prone position and allowed to recover from tranquilization.

During the four hour baseline period, the animals will receive an intravenous infusion of normal saline with 44 mEl/L of $NaHCO_3$ at a rate of 10 cc/kg/hr. This infusion will insure a constant basal urine output. A primary dose of PAH (40 mg/kg) and Inulin (75 mg/kg) will be given. This will be followed by a constant infusion of 0.5% PAH and 1.0% Inulin in saline, at 6 cc/kg/hr for the remainder of the experiment. At 60 and 75 minutes after priming, arterial and renal vein samples and fifteen minute urine collections will be obtained for the course of the study.

Every 30 minutes thereafter, blood samples will be taken. Beginning with the first 30 minute period, a 50 cc bolus of hemoglobin solution (6 gms %) will be given every 15 minutes.

This will continue until a volume of stroma free hemoglobin solution (50% of the animals calculated circulating blood volume) has been infused. At the end of this period, the animals will be sacrificed and autopsied.

Renal Clearance Determinations: Plasma and urine concentrations of spectrophotometric techniques. The glomerular filtration rate will be determined by:

$$GFR = \frac{\text{(Urinary Conc. of Inulin) (Urine Volume)}}{\text{(Plasma Conc. of Inulin)}}$$

The renal blood flow will be determined by:

$$\text{Renal Plasma Flow} = \frac{\text{(Urinary Conc. of PAH) (Urine Volume)}}{\text{(Renal Art. Conc. of PAH - Renal Vein Conc. of PAH)}}$$

$$\text{Renal Blood Flow} = \frac{\text{Renal Plasma Flow}}{\text{1 - Hematocrit}}$$

The Free Water Clearance will be determined by:

$$FWC = \frac{\text{(Urine Osmolarity) (Urine Volume)}}{\text{(Plasma Osmolarity)}} - \text{Urine Volume}$$

The urine and plasma osmolarities will be determined by freezing point depression.

Renal A-V Shunting: To test for renal A-V shunting, blood will be drawn anerobically for simultaneous blood gas determinations.

3. EFFECT OF STROMA FREE HEMOGLOBIN ON BRAIN OXYGENATION

In order to evaluate the effect of a leftward shifted P_{50} on the brain, the following study will be undertaken:

Adult male baboons will be tranquilized and femoral venous and arterial cutdowns will be performed. One jugular vein and one carotid artery will also be cutdown. The animals will be allowed to equilibrate. Then they will be paralyzed with d-tubocurarine, intubated, ventilated and their arterial Po_2 and Pco_2 and pH will be adjusted, and baseline measurements taken.

The animals will be exchange transfused with stroma free hemoglobin solution. At decrements of 10% in the hematocrit the following measurements will be taken from the arterial, venous, carotid and jugular routes; pH, Pco_2, Po_2 serum lactates, O_2 contents. Through an indwelling catheter cerebiospinal fluid will be sampled and pH, Pco_2, Po_2, lactate levels will be measured. EEG's will be taken each measurement period after the animal has stabilized.

A control group of Dextran-75 exchange transfused animals will be handled in an analogous manner and exchange transfused to a hematocrit of 20% (6 gms % hemoglobin).

When the stroma free hemoglobin animals reach 0% hematocrit (6 gms % hemoglobin) the above measurements will be taken hourly for three hours.

Following the experimental period the animal will be sacrificed, the horn of hippocampus removed, and sent to pathology for sectioning and viewing.

4. COLLABORATIVE STUDY USING HEMOGLOBIN SOLUTION WITH A NORMALIZED P_{50}

Dr. Thomas Zuck, Chief of the Blood Research Division at Letterman Army Institute of Research in San Francisco, California, has agreed to supply us with a hemoglobin solution prepared by a technique different than ours. The P_{50} of this hemoglobin solution is more normal (17-19 mm Hg) than our preparation. We have agreed to use our baboon model to test this hemoglobin solution as soon as the logistics of supplying large amounts of this preparation can be worked out. We anticipate a start up for the project sometime during this year.

The first experiments using this solution will be simple exchange transfusions measuring the same parameters as measured in our earlier work (refer section A, studies completed this year; of this proposal).

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